Journal of Research on the Lepidoptera 31(3-4):213-232, 1992

The influence of larval age and ant number on myrmecophilous interactions of the African Grass Blue butterfly, *Zizeeria knysna* (Lepidoptera: Lycaenidae)

Konrad Fiedler and Dorthe Hagemann

Zoologie II, Biozentrum der Universität, Am Hubland, D-97074 Würzburg, Germany

Abstract. Interactions between myrmecophilous *Zizeeria knysna* larvae and *Lasius flavus* ants were quantitatively studied in laboratory experiments. Larvae delivered secretions from their dorsal nectar organ (DNO) more frequently in the initial 3-minute interval of an interaction than later on. Tentacle eversions were likewise more common at the beginning of interactions. Non-feeding prepupal larvae secreted significantly more droplets than feeding fourth instars. Actual tending levels differed between larvae tested with 5 (2.6-3.1 ants per larva) or 15 ants (5.3 ants/larva), respectively. Secretion rates increased with tending level (5-ant trials: feeding L4 larvae 5.5 DNO droplets/h, prepupae 16.4 droplets/h; 15-ant trials: feeding L4 9.5 droplets/h, prepupae 25.5 droplets/h). Secretion droplets averaged 0.2 mm in diameter (volume 0.004 µl). From these data, a model is developed to estimate lifetime DNO secretion amounts of individual larvae. Estimates range from 1.3-4.7 µl per larva in 5 d, representing approximately 0.2-0.7 mg carbohydrates with a physiological energy equivalent of 3.4-12 J. Hence, *Z. knysna* larvae provide only a marginal food reward for attendant ants, suggesting that myrmecophily is a low-cost life-history strategy in that butterfly species.

Key Words: mutualism - symbiosis - caterpillars - strategic behavior - energetic investment - Formicidae - Lycaenidae

Introduction

Interactions between immatures of butterflies and ants, termed myrmecophily, are widely known from a broad range of lycaenid and riodinid species (see reviews by Malicky 1969; Cottrell 1984; Pierce 1989; Fiedler 1991). Much work has concentrated on the description of individual life-cycles, on the structure and function of myrmecophilous organs, and on the ecological outcome of myrmecophily (mutualism, parasitism). In contrast, fewer studies have focused on detailed quantitative studies of the relevant behaviors. Such studies either attempted to quantify myrmecophilous interactions in the light of interspecific comparisons (Fiedler 1991, Ballmer & Pratt 1991), or they experimentally elucidated phenomena like the release of food recruitment in tending ants (Fiedler & Maschwitz 1989), the secretory capacity of individual larvae (Fiedler & Maschwitz 1988a), the influence of larval food on the

Paper submitted 25 July 1994; revised manuscript accepted 15 November 1994.

expression of myrmecophily (Fiedler 1990, Baylis & Pierce 1991), or on conditional factors regulating secretory behavior (Leimar & Axén 1993). With the exception of the last mentioned work, all studies assumed that secretion rates observed in experiments are more or less representative for the populations or species under investigation.

The energetic investment of lycaenid larvae in their symbiosis with ants may, however, be plastic in response to the actual needs. For example, Leimar & Axén (1993) showed that larvae of the facultatively myrmecophilous species *Polyommatus icarus* (Rottemburg, 1775) delivered more secretion droplets from their nectar organ when subjected to a simulated attack or when tended by two instead of one *Lasius flavus* (Fabricius, 1781) worker ants. Further increase in the number of tending ants did not add to secretion rates. In addition, the intensity of myrmecophily often increases with progressive larval development (e.g. Malicky 1969, Fiedler 1989), although Leimar & Axén (1993) observed no significant correlation between body mass and secretion rates among fourth instars of *P. icarus*. Wagner (1993) demonstrated significant weight losses in non-feeding prepupal larvae of a Nearctic facultative myrmecophile, *Hemiargus isola* (Reakirt, [1867]). Her result points to particularly intensive and energetically costly interactions with ants in the prepupal phase.

We here present a quantitative laboratory study on myrmecophilous interactions between larvae of *Zizeeria knsyna* (Trimen, 1862) and the ant *Lasius flavus*. Specifically, we address the following questions: 1) Are the secretions from the dorsal nectar organ (DNO) delivered at a constant rate or is there a temporal pattern in the secretory behavior? 2) Are the secretion rates of feeding mature larvae equal to those of non-feeding prepupal larvae? 3) Does the number of tending ants influence the outcome of larva-ant interactions? 4) Is larval myrmecophily correlated with body mass and how are the various myrmecophilous behaviors correlated with each other? 5) Finally, we try to estimate the lifetime investment in nectar-like secretions of individual larvae of *Z. knysna*.

Material and methods

Study organisms

The African Grass Blue, *Zizeeria knysna* is a small butterfly distributed from the Canary islands and the Iberian Peninsula southwards throughout most of Africa, including Madagascar and the Mascarene islands, eastwards extending to Arabia. The species is polyvoltine. It occurs in open, xeric habitats, and the larvae feed on a variety of plants, notably various genera of Fabaceae, but also on members of Amaranthaceae, Chenopodiaceae, Oxalidaceae, Zygophyllaceae and Euphorbiaceae. In addition, oviposition has been observed on Malvaceae (Schurian 1994). There are 4 larval instars, with older larvae facultatively tended by ants (see Clark & Dickson 1971 for a detailed illustrated description of the basic life cycle). Tending ants have rarely been specified. Schurian (1994) recorded a *Pheidole* species (Myrmicinae) from the Canary islands. Further records, such as *Tapinoma melanocephalum* (Dolichoderinae: Warnecke 1932/

33), refer to the related butterfly, *Z. karsandra* (Moore, 1865), whose status as a distinct species has been subject to controversy until recently.

For our experiments, we used laboratory-bred offspring of females caught on Gran Canaria. Butterflies were kept in plastic cages in a greenhouse (see Schurian 1989, for details on the breeding method). The laboratory stock was maintained for 5 generations throughout the year 1993 using inflorescences and young foliage of *Medicago sativa* (Fabaceae) as the main larval food. Experiments were conducted with members of the 4th and 5th generation between September and November 1993. To control for possible effects of larval diet (Fiedler 1990, Baylis & Pierce 1991), we fed all experimental animals invariably with young foliage of *M. sativa*. Larvae were reared in an environmental chamber at 25.5 °C and 15:9 h L:D cycle. They were kept in transparent plastic vials (250 ml) lined with moist filter paper. *Ad libitum* amounts of freshly cut terminal foliage of *M. sativa* were provided daily, and the larvae were transferred to a new rearing vial every day to minimize the risk of diseases.

Lasius flavus (Formicinae) is a common subterranean ant species of the Palearctic region. It mostly occurs in open grassland or heaths, but also colonizes forests (Kutter 1977). *L. flavus* avoids truly xeric habitats and therefore probably rarely, if ever, co-occurs with *Z. knysna*, although the distributions of both species overlap on the Iberian Peninsula and in northwestern Africa. The diet of *L. flavus* ants mainly consists of the honeydew of root aphids. Furthermore, aphids are eaten in large quantities to obtain proteins (Pontin 1978). Due to their food specialization, *L. flavus* ants show intensive trophobiotic behavior even under laboratory conditions and avidly tend lycaenid larvae and pupae (e.g. Fiedler 1990, 1991, Leimar & Axén 1993). Therefore, this ant species is very suitable for laboratory studies on lycaenid myrmecophily, although associations between *L. flavus* worker ants and lycaenid immatures have rarely been observed in nature (Fiedler 1991).

Ant colonies were kept at laboratory temperatures of approx. 20-23 °C under ambient light conditions in large earth nests, which were maintained in plastic arenas (size 64 cm × 44 cm × 12 cm) with a bottom of plaster of Paris. Sidewalls were smeared with Fluon to prevent ants from escaping. The nests were sprayed daily with water to adjust humidity, and food (honey-water and cut cockroaches) was provided as needed. For our experiments we used three ant colonies originating from northern Bavaria.

Experimental procedure

Experiments were conducted in plastic arenas (10 cm × 10 cm × 6 cm) with a bottom of plaster. The bottom was kept moist during all trials. For experiments, either 5 or 15 foraging workers of *L. flavus* were taken while on their way to a feeding place in the foraging area of the nest arenas and were carefully transferred into a test arena with the help of a brush. Disturbance of ants due to handling was minimized and another 5 min allowed before a single test larva was placed in the center of the test arena. After that time period the alarm behavior of the ants had subsided. Beginning with the first encounter between an ant and the larva, we recorded the behavioral interactions for 15 min. Observations were made under a Zeiss stereomicroscope at ten-fold magnification with normal daylight between 9:00 h and 15:00 h local time. The arena was rotated from time to time to eliminate possible effects of directional illumination on ant activity.

The following events were counted every 30 seconds: a) the number of ants in

immediate physical contact with the larva; b) the number of DNO secretion droplets delivered during that time interval; and c) the number of eversions of the tentacle organs (TOs). Total duration of contacts between ants and the test larva were recorded with a stop-watch to the nearest second. At the end of each experiment, the larva was weighed to the nearest 0.1 mg (Sartorius Basic BA 61 balance).

Each set of worker ants was used for a maximum of three subsequent experiments to avoid possible effects due to a drop in ant activity if kept in isolation from their nestmates for longer periods. In a large series of earlier experiments (Burghardt & Fiedler, unpubl.) we have established that no adverse effects occur if *L. flavus* are kept away from their colony for up to 1 h. At least 5 min elapsed between the experiments.

Two classes of larvae were used in experiments. "Feeding larvae" refers to animals that were well within the fourth (= final) instar and had not yet left the foodplant to settle down for pupation. Feeding L4 larvae in our tests ranged from 25.7- 53.0 mg (wet weight) and were all near their larval peak body mass. "Non-feeding prepupal larvae" denotes those which had stopped feeding. Such larvae had mostly left the hostplant to settle down among the filter papers, but had not yet spun a silk girdle. They all showed a characteristic transformation of color: their markings became indistinct and the overall appearence was transparent and "glossy". Non-feeding prepupal larvae are still able to crawl and their myrmecophilous organs remain functional. Wet weights of non-feeding prepupae tested ranged from 26.1-51.2 mg. After one day, the true immobile girdled prepupa is formed, which is no longer able to evert the tentacle organs.

Larval sex discrimination was not attempted, since sexual weight dimorphism in our laboratory cultures was generally low. Any larva was tested only once per day and at most twice per lifetime (once as a feeding larva, again as a non-feeding prepupa). A few larvae in each series were examined in only one of these phases.

Quantitative evaluation of results

Attractiveness, or actual tending level, was calculated from data recorded for each individual larva (defined as the arithmetic mean of the number of tending ants throughout the experiment, i.e. across 30 census points). In addition, we calculated the total number of secretion droplets delivered per experiment and the sum of tentacle eversions. To examine the time course of larva-ant interactions we subdivided each experimental period into five 3-min intervals. Since this analysis revealed a distinct difference between the first 3-min interval and the subsequent intervals (see below), we also calculated the number of secretion acts and tentacle eversions of each experimental larva for the final 12 min of a trial.

All data were then subjected to statistical analysis. Comparisons between the larval age classes or between the experimental series with different ant numbers were computed using the non-parametric U-test of Mann & Whitney, while comparisons between the time intervals within experiments were made using Wilcoxon's matched-pairs signed-ranks test. Spearman rank correlations between myrmecophily parameters and larval weight were likewise calculated (Sachs 1992).

Results

Temporal patterns of larva-ant interactions

Regardless of larval age or ant number, all experiments with *Z. knysna*

larvae revealed similar temporal patterns of myrmecophilous behaviors and interactions. DNO secretions occurred significantly more often in the first 3-min interval than in the four subsequent intervals of the experiments. This was true for feeding larvae (Fig. 1A) and non-feeding prepupae (Fig. 1B) in experiments with either 5 or 15 *L. flavus* worker ants (Wilcoxon-test, $p < 0.02$ for all comparisons between first and second 3-minute experimental interval). On average, 1-2 droplets were delivered by feeding larvae, and 2-3 by non-feeding prepupae, in the initial three minutes. This compares to 1-2 droplets (feeding L4) or 3-5 droplets (prepupae) in the subsequent 12 min.

Virtually the same pattern occurred with the TO eversions. Feeding larvae everted their TOs significantly more often in the first 3 min than later (Wilcoxon-test, $p < 0.01$ for experiments with both 5 and 15 ants), and in the final 9 experimental minutes TO eversions were very rare (Fig. 2A). The same was observed with non-feeding prepupae (Wilcoxon-Test, $p < 0.01$), but the effect was delayed in the experiments with 15 ants to the third 3-min interval (Fig. 2B). Overall, TO eversions occurred more frequently in the prepupae during the final 9 experimental minutes.

The attractiveness of larvae to ants remained stable throughout the course of the experiments. All larvae were almost constantly tended from their first encounter with ants. Total tending times were 12:45-15:00 min in experiments with five *L. flavus* ants (only five feeding L4 and four prepupae had association times shorter than 15:00 min), and 13:27-15:00 min in trials with 15 ants (three prepupae had association times lower than 15:00 min). Within 1-2 min after the first encounter, the number of tending ants in all experiments reached the average level. Rarely there was a further slight increase, but never a distinct drop, in the number of tending ants with time.

Comparison between feeding mature larvae and non-feeding prepupae

There was a distinct difference in DNO secretion rates between feeding larvae and prepupae (Fig. 3). During both experimental series with either 5 or 15 ants, prepupae produced much more secretion droplets than feeding fourth instars (U-test, $p < 0.002$, with or without the first 3 min of each experiment being included).

Due to the numerical preponderance of TO eversions in the initial 3 min of each experiment, the total frequency of TO eversions throughout the 15-min trials showed no significant differences between the two larval age classes ($p > 0.20$ for eversion rates in 15 min, with both 5 and 15 ants). When the initial 3 min were deducted, a significant difference emerged in the 15-ants series: prepupal larvae everted their TOs significantly more often than feeding fourth instars (U-test, $U_{19;20} = 114$, $p < 0.05$). In the 5-ants series, a similar, albeit non-significant difference between the two age classes occurred.

In experiments with 5 ants, the actual tending level (mean number of tending ants per larva) increased slightly, but significantly, from the

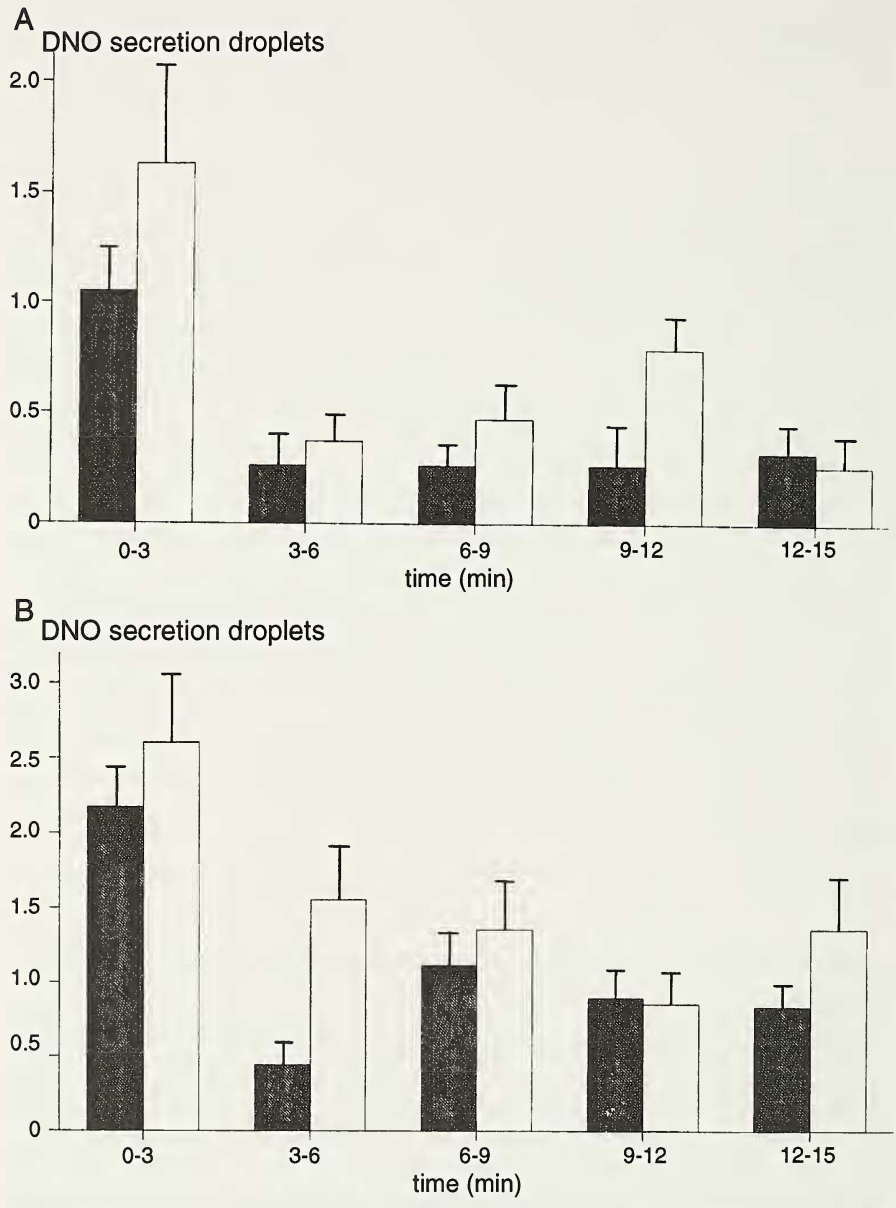

Fig. 1: Temporal pattern of DNO secretion acts observed in experiments with larvae of *Zizeeria knysna*. Given are means + standard errors for five successive 3-min time intervals. Hatched bars: with 5 *Lasius flavus* ants; white bars: with 15 ants. A): feeding mature fourth instars (n = 19 with 5 ants; n = 20 with 15 ants); B): non-feeding prepupae (n = 18 with 5 ants; n = 20 with 15 ants). Initial secretion rates are significantly higher than in subsequent 3-min intervals intervals (Wilcoxon-test, $p < 0.05$).

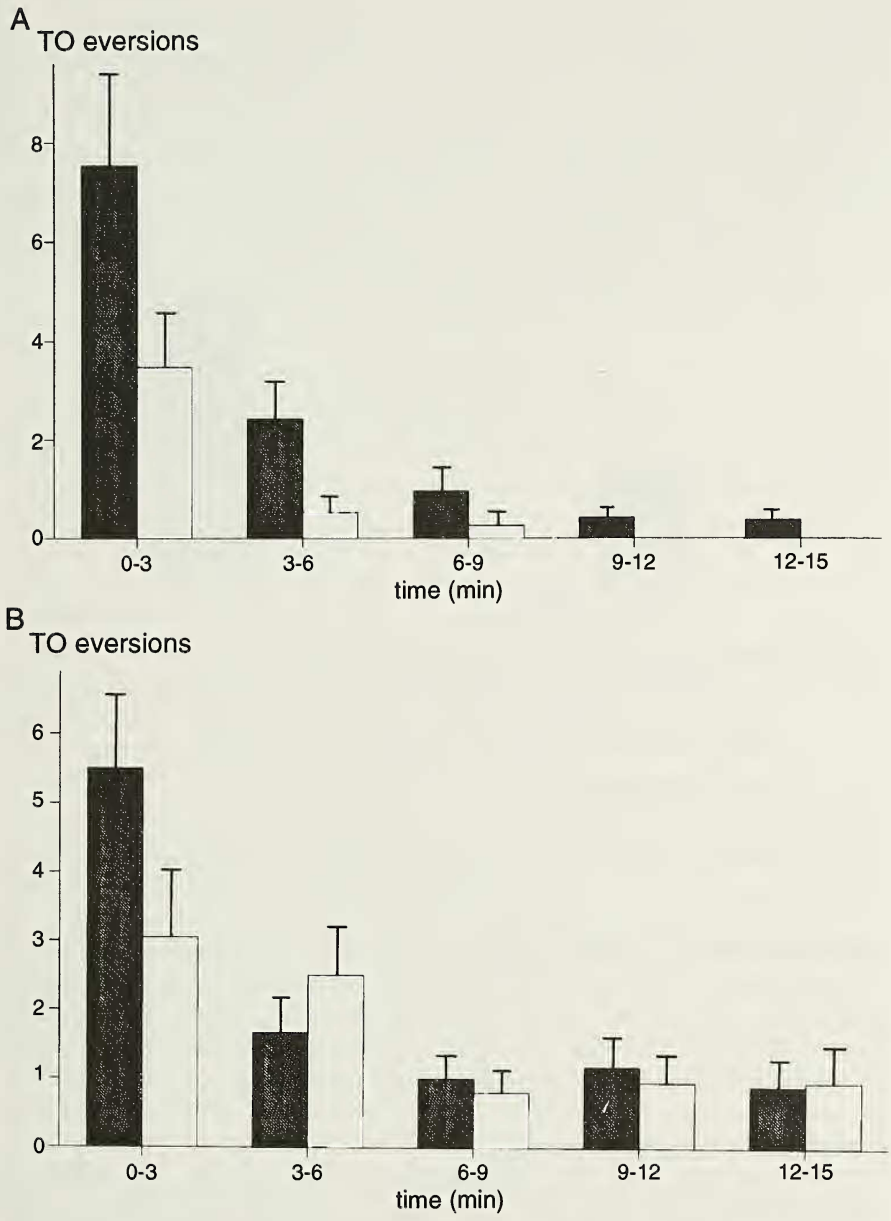

Fig. 2: Time course of TO eversion rates (means + S.E.) in *Z. knysna* larvae. Hatched bars: with 5 *L. flavus* ants; white bars: with 15 ants. A): feeding mature fourth instars (n = 19 with 5 ants; n = 20 with 15 ants); B): non-feeding prepupae (n = 18 with 5 ants; n = 20 with 15 ants). Initial eversion rates are significantly higher than in subsequent 3-min intervals (Wilcoxon-test).

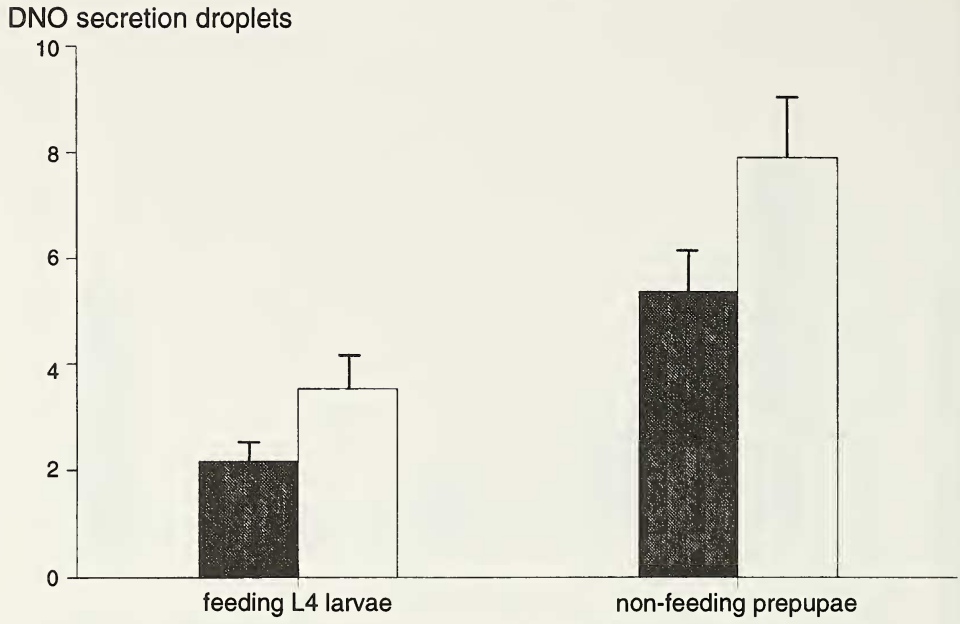

Fig. 3: Total number of DNO secretion droplets (means + S.E.) delivered in 15-min experimental intervals by larvae of *Z. knysna*. Hatched bars: with 5 *L. flavus* ants (n = 19); white bars: with 15 ants (n = 20). Differences between feeding larvae and non-feeding pupae, as well as between 5-ants and 15-ants trials are all statistically significant (U-test, $p < 0.05$).

feeding (2.63 ants/larva) to the non-feeding prepupal phase (3.13 ants / larva; $U_{18;19} = 100$, $p < 0.05$). In the parallel series with 15 ants, larvae received an actual tending level of 5.35 ants/larva already during the feeding phase and this did not change with the transition into the prepupal stage (5.27 ants/larva). Hence, prepupal larvae attract a larger group of worker ants than still feeding mature larvae; however, under our experimental conditions an upper physiological limit ("saturation") is reached at an average of roughly 5 ants per larva.

The influence of ant number

DNO secretions occurred more frequently among experiments with 15 ants, but this difference was only marginally significant for feeding larvae ($U_{19;19} = 133$; p (1-tailed) < 0.10) or non-feeding prepupae ($U_{18;19} = 127$; p (1-tailed) < 0.10) (Fig. 3). The same statistical trend was observed when the secretion events of the initial 3-min intervals were removed.

No consistent result was obtained with respect to TO eversions. Feeding larvae everted their TOs more frequently in experiments with fewer ants present ($p < 0.02$), but this difference largely disappeared in the prepupal stage ($p > 0.20$).

The actual mean tending level increased from 2.63-3.13 ants/larva in

the 5-ant trials to 5.27-5.35 ants/larva in the 15-ant experiments. A threefold increase in the number of available mutualists thus resulted only in an increase of the tending level by a factor of 1.7-2.0. In the 5-ant series, larvae or prepupae attracted on average 52-62% of their available mutualists, whereas in the 15-ant trials only 35% of the ants actually tended the lycaenid immatures. Maximum tending levels were, however, much higher. Two prepupal larvae attracted 8.29 and 9.63 ants, respectively (averaged over the 15 min period). These two animals were tended by 10-11 ants over several minutes and were then literally covered.

Rank correlations

Neither at the feeding stage nor in the prepupal phase was the DNO secretion rate significantly correlated with body mass (r_s values ranged from -0.006 to 0.318, $p > 0.10$). We also failed to detect significant correlations between the frequency of TO eversions and DNO secretion rates (r_s ranging from -0.244 to 0.017, $p > 0.17$), or between TO eversion rates and actual tending levels (r_s between -0.304 and 0.154, $p > 0.12$). Correlations did, however, occur between actual tending level and DNO secretion rates (feeding larvae: $r_s = 0.336$, $p = 0.093$ (with 5 ants); $r_s = 0.381$; $p = 0.066$ (with 15 ants); prepupae (with 15 ants): $r_s = 0.535$, $p < 0.01$). Similar correlations were obtained, when the DNO secretion data from the initial 3 min of each trial were removed.

These results suggest that DNO secretion and TO eversion rates are independent from one another and that body mass plays at most a minor role in the myrmecophily of *Z. knysna* immatures. A larger ant guard is somewhat more effective in stimulating more frequent DNO secretions, but this relationship is far from being close.

Estimates of individual lifetime production of DNO secretions

Based on our experimentally established figures for average DNO secretion rates of *Zizeeria knysna* larvae, we here develop a model to estimate the total lifetime investment of individual larvae in these secretions. For this purpose, we assume that a) our experimental values of secretion rates are representative, and b) secretion rates remain largely constant once a larva-ant association is established. Therefore, we only use the average secretion rates from the final 12 min of our experiments because at the beginning of larva-ant interactions secretions occur more frequently for a short period of time (see above). Accepting these premises, hourly DNO secretion rates are as follows:

- with 5 ants per trial (i.e. actual tending level 2.63-3.13 ants/larva): feeding L4 1.1 droplets/12 min = 5.5 droplets/h; prepupae 3.3 droplets/12 min = 16.5 droplets/h;
- with 15 ants (i.e. actual tending level 5.3 ants/larva): feeding L4 1.9 droplets/12 min = 9.5 droplets/h; prepupae 5.1 droplets/12 min = 25.5 droplets/h.

In our laboratory culture the active feeding period of fourth instars

lasted 4 days and the larvae remained about one day in the non-feeding prepupal phase. Clark & Dickson (1971) recorded a developmental time of 6-7 days for the entire fourth instar in South Africa, hence our laboratory animals grew somewhat faster than under subtropical field conditions.

Furthermore, we assume that a *Z. knysna* larva is tended by ants for at least 8 h daily throughout the fourth instar. For comparison, we also calculate secretion rates under the assumption of a permanent (24 h daily) ant-association. We assume that the period of increased secretion rates within the prepupal phase does not exceed 8 h because the non-feeding prepupa then becomes fully immobile and the DNO non-functional. Field data on tending levels of *Z. knysna* are not yet available, but observations on many other facultatively myrmecophilous lycaenids suggest that it is realistic to postulate 8-24 h daily tending by 2-5 ants per larva. Our model hence provides upper and lower limits for lifetime DNO secretion amounts.

Under these assumptions, a *Z. knysna* L4 in our 5-ant trials would secrete 308 (8-hour ant association) to 660 (permanently ant-tended) droplets from its DNO. The respective values for the 15-ant trials are 508 (8 h) to 1116 droplets (24 h).

The diameter of secretion droplets was determined using a calibrated eye-piece on the stereomicroscope. DNO droplets of *Z. knysna* larvae measured 0.233 ± 0.061 mm in diameter (n = 6, range 0.15-0.30 mm), corresponding to a mean droplet volume of 0.00662 µl. For the following calculations, we used an average droplet diameter of 0.2 mm (volume 0.00419 µl) to avoid overestimation. The lifetime secretion volumes of individual *Z. knysna* larvae can thus be estimated to range from 1.3-2.8 µl in 5-ant trials and from 2.1-4.7 µl in 15-ant trials.

Data on the energy content of DNO secretions are unavailable for *Z. knysna* or any closely related lycaenid butterflies. In the facultatively myrmecophilous European species *Polyommatus (Lysandra) hispanus* (Herrich-Schäffer, 1852) and *P. icarus*, the secretions contain approximately 15 % carbohydrates (Maschwitz et al. 1975). If we assume a similar composition of DNO secretions for *Z. knysna*, then the individual lifetime secretion volumes are equivalent to 0.2-0.42 mg (5 ants) or 0.32-0.71 mg carbohydrates (15 ants).

The mean dry weight ± SD of adult specimens (males and females pooled) from our laboratory culture was 2.78 ± 0.71 mg (range 1.2-4.5 mg, n = 43). In relation to the average adult weight, the estimated carbohydrate content of larval DNO secretions is equivalent to 7.2-15.1 % (5-ant trials) or 11.5-25.5 % (15-ant trials).

Discussion

Temporal patterns of interactions

Interactions between *Zizeeria knysna* larvae and ants show a clear

temporal pattern: DNO secretions as well as TO eversions occur most frequently at the very beginning of a myrmecophilous association and rapidly decrease to a rather constant and much lower level. This general pattern occurred in both age classes and with both ant densities tested. Similar results have been obtained with additional Palearctic lycaenid species *(Polyommatus candalus* (Herrich-Schäffer, [1851]): Fiedler et al. 1994; *Polyommatus icarus*: Burghardt 1994; *Aricia agestis* ([Denis & Schiffermüller], 1775): Hummel 1994; *Polyommatus daphnis* ([Denis & Schiffermüller], 1775), *P. coridon* (Poda, 1761), *Glaucopsyche alexis* (Poda, 1761): Fiedler, unpubl.). The phenomenon, however, is not universal. In larvae of *Celastrina argiolus* (Linnaeus, 1758) tested in exactly the same manner, the clumped occurrence of DNO secretions and TO eversions at the beginning of experimental interactions was not apparent (Burghardt 1994).

Three mechanisms could be responsible for this effect: a fixed "physiological" reaction to empty a full DNO reservoir; a response to disturbance and handling; or an increased initial investment to intensify ant-associations from the very beginning of an interaction. All experimental larvae had not been "milked" by ants for one or more days (and some never before in their life) and hence probably had well filled secretion reservoirs. One might assume that larvae at first deliver, in a kind of "fixed action pattern", the entire reservoir content at a relatively high rate, whereas later secretion acts can only be continued at a rate equal to the physiological capacity of secretion supply replacement. Then, the high initial secretion rate would be a non-adaptive epiphenomenon.

However, most *Z. knysna* larvae secreted only 1-3 DNO droplets in the initial 3 min, which is most probably less than their reservoirs' capacity. In *Polyommatus icarus* and *Aricia agestis* we estimated the DNO reservoir volume of larvae using Malicky's (1969) histological data on the size of DNO gland cells (Fiedler, Burghardt & Hummel, unpubl.). According to these data, a well-filled DNO reservoir should contain 10 droplets or more. Furthermore, high initial rates of TO eversions accompanied the enhanced DNO secretion rates. It is therefore unlikely that *Z. knysna* larvae in our experimental setup really delivered all their stored secretion resources, when interactions with ants commenced.

Alternatively, the larvae may have responded to the inevitable disturbance and handling when introduced into the experimental arena. Leimar and Axén (1993) have shown that lycaenid larvae may respond to tactile disturbance with a temporary increase in DNO and TO activity. According to their data, and our own experiments with *Polyommatus icarus* and *P. coridon* (Fiedler, unpubl.), the effect of mild tactile disturbance is in the range of one additional secretion droplet. This is exactly the increase we found at the beginning of experiments with *Z. knysna*, while in *P. icarus* and *Aricia agestis* the difference between initial secretion rates per 3-min interval and subsequent 3-min intervals was

more distinct (2-4 additional droplets per 3 min: Burghardt 1994, Hummel 1994).

We suggest that the high initial secretion rate, accompanied by high TO activity, is an evolved adaptive behavior, although response to tactile disturbance may well be involved as a proximate factor. The very beginning of a larva-ant interaction is decisive for the subsequent stability of such an association. If a larva immediately provides an attractive food resource, it will be tended more constantly and may also induce the scout ant to recruit additional nestmates. The secretory behavior of *Z. knysna* larvae matches the "enticement and binding" strategy described by DeVries (1988). Increased initial activity of the TOs most likely serves the same function. Although previously debated controversially (Malicky 1969),TO eversions alert and activate tending ants, and their role in stabilizing larva-ant associations has been demonstrated at least in certain cases (Fiedler & Maschwitz 1988b, Ballmer & Pratt 1991).

Hence, temporal patterns of myrmecophilous interactions in *Z. knysna* larvae indicate what has been termed "strategic behavior" by Leimar & Axén (1993): caterpillars initially make a considerable effort (up to 9 droplets per 3 min) to establish an ant-association, but subsequently reduce this energetic investment to minimize costs. This finding has an important consequence. When lycaenid-ant interactions are studied in laboratory assays, the initial DNO secretion or TO eversion rates may be misleading. Observations should last until more or less stable "steady-state" conditions are reached. Experiments of short duration (e.g. 5-min trials by Ballmer & Pratt 1991) therefore become difficult to evaluate with respect to their ecological relevance.

Increased myrmecophily in prepupae: adaptive trait or physiological epiphenomenon?

The investment of *Z. knysna* larvae in myrmecophily does not increase steadily with larval growth. In a couple of experiments with half-grown fourth instars of *Z. knysna* (6 with 5 ants, 3 with 15 ants; data not shown), DNO secretion and TO eversion rates were identical to the figures obtained with mature feeding L4 larvae. Furthermore, there was no positive significant correlation between larval weight and DNO secretion rates. Leimar & Axén (1993) and Burghardt (1994) likewise found that secretion rates were not correlated with either larval weight or age in feeding *P. icarus* larvae. Surprisingly, however, there is a rapid increase in secretion rates of *Z. knysna* immatures with the transition from the feeding to the non-feeding prepupal phase. Parallel effects have been observed in other Polyommatini species (*Polyommatus icarus, P. candalus, P. coridon, Aricia agestis*: Burghardt 1994; Hummel 1994; Fiedler et al. 1994 & unpubl.). The results of Wagner (1993) also indicate a particularly high investment into myrmecophily by prepupal *Hemiargus isola*.

Two possible mechanisms can explain this increase in prepupal secre-

Journal of Research on the Lepidoptera 31(3-4):233-277, 1992

Survey of Adult Morphology in Nystaleinae and Related Neotropical Subfamilies (Noctuoidea: Notodontidae)

S. J. Weller

Dept. of Entomology, Univ. of Minnesota, St. Paul, MN 55108

Abstract. Based on a comparative study of 71 neotropical and 10 palearctic genera, morphological trends in Nystaleinae were ascertained. Over half the nystaleine species were examined (135 of 253). A diverse sample of neotropical Heterocampinae (27 of 37 neotropical genera, 46 species) and Hemiceratini (7 of 11 genera, 15 species) was also surveyed. Additional palearctic and nearctic notodontid species were examined in the more general study.

Survey results are presented along with illustrations of cephalic, thoracic and abdominal structures. Previous interpretations of internal tympanal structures are discussed, and sexually dimorphic structures described and illustrated. A checklist of nystaleine genera is provided. New morphological terms are proposed and synonyms are noted.

Introduction

The family Notodontidae (Lepidoptera: Noctuoidea) consists of approximately 3,200 species worldwide (Holloway, Bradley, and Carter, 1987). The greatest diversity, over 1300 species, occurs in the New World tropics. Adults are usually heavy-bodied moths with pilose vestiture and cryptic coloration. Wingspans range from 127 mm (*Anurocampa mingens* Herrich-Schäffer, female) to as small as 20 mm (*Talmeca curtoides* Dognin, female). Notodontid larvae are notable for their often bizarre morphology, and some possess unique chemical defenses (cyanic acid, formic acid, and other ketones: Blum, 1981). Many species undergo striking ontogenetic changes between larval stadia, particularly in the Heterocampinae (Packard, 1895; Godfrey and Appleby, 1987). Notodontid larval host plants include both monocots and dicots (the majority on woody dicots), and larvae are usually either monophagous or oligophagous (Miller, 1992).

Little descriptive morphology is available for neotropical Notodontidae. Notodontid morphology is either discussed very generally based on few examples at the family or superfamily level (e.g., Brock, 1971; Richards, 1932), or discussed for only a few species within a faunal treatment or generic revision (e.g., Forbes, 1939a, 1948; Franclemont, 1948; Thiaucourt, 1975, 1980, 1985, 1987). Only recently has a comparative study among subfamily representatives been published (Miller, 1991).

In this paper, I describe and illustrate cephalic, thoracic and abdominal structures found in many notodontids, concentrating on Nystaleinae

Paper submitted 21 March 1994; revised manuscript accepted 21 February 1995.

(Tables 1, 2). The checklist of nystaleine genera (Table 1) is assembled from Weller (1989). Many of the following descriptions are new, because previous workers have concentrated on nearctic taxa. Most of my findings concern Nystaleinae, but I also comment on other taxa that illustrate character novelties or important character distributions. Previous interpretations of tympanal structures are discussed, and putative, pheromone-producing structures in males and females are described and illustrated. A summary of morphological terms and proposed equivalents is included.

Materials and Methods

Preparation of specimens

Body parts (abdomens, appendages) or entire specimens (except wings) were softened in hot 10% KOH, then cleaned in several rinses of 40% ethanol. Genitalia were stained with either chlorozol black (dissolved in 20% ethanol), or with chlorozol black followed by saffranin (dissolved in 95% ethanol). Stained preparations were positioned, dehydrated, and mounted in either balsam or euparol. The membranous pleats of male genitalia trap water. Best dehydration results were obtained when positioned genitalia were left in sealed dissecting dishes of 95% ethanol for 4-12 hours. Antennae, labial palpi and legs were treated similarly, except that they were not stained. Wings were bleached, stained with Eosin Y, and mounted in balsam.

Softened whole-body preparations were prepared by first removing the abdomen. Either the head and prothorax were removed as a unit, or just the metathorax was removed. Once the viscera and scales were removed, preparations were stained with chlorozol black to enhance membrane contrast with the cuticle.

To examine the recessed tympanal membrane, I rotated the body wall so that the venter was 10 to 30 degrees above horizontal. Different preparations were used to expose the tympanum. In some, the isolated metathorax was entire. In others, midline dorsal and lateral cuts were made. The most satisfactory tympanal preparations resulted when the first abdominal tergum was left connected to the metathorax. In Table 3, the number of preparations is summarized. A complete list of species, sex, dissection numbers and type of dissection (e.g., whole body, genitalia) is available in Weller (1989).

Sources of specimens

Material from the following collections was examined. Abbreviations follow Heppner and Lamas (1982): AMNH, American Museum of Natural History, New York (F.H. Rindge); BMNH, British Museum (Natural History), London (A. Watson); CAS, California Academy of Sciences, San Francisco (P.H. Arnaud); CMNH, Carnegie Museum of Natural History, Pittsburgh (J.E. Rawlins); CNC, Canadian National Collection, Ottawa, Canada (J.D. Lafontaine); CU, Cornell University, Ithaca, New York (J.K. Liebherr); DJ, D. Janzen, private collection, Univ. of Pennsylvania; LACM, Los Angeles County Museum, California (J.P. Donahue); MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts (J. Carpenter); NMNH, National Museum of Natural History, Smithsonian Institution, Washington, D.C. (R. Poole, R.K. Robbins); SJW, S.J. Weller preparation, University of Minnesota Insect Collection; UMO,

Table 1. Checklist of nystaleine genera examined (modified from Weller, 1989). Descriptions of new genera, and justifications of other taxonomic changes are given in Weller (1989, in prep.). Type species of new genera are provided below.

NYSTALEINAE: NYSTALEINI
Ankale Weller, NEW GENUS
Lepasta, of authors [not Möschler, 1878]
grammodes Felder, 1874 [Nystalea] NEW COMBINATION
conspicua Butler, 1878a [Lepasta] NEW SYNONYMY
Antiopha Schaus, 1901
Tachuda Schaus, 1901, NEW SYNONYMY
Naduna Schaus, 1901, NEW SYNONYMY
Bardaxima Walker, 1858b
Gisara Schaus, 1901 NEW SYNONYMY
Gozarta Walker, 1869
Calledema Butler, 1875
Pseudantiora Kirby, 1892 REVISED STATUS
Dasippia Draudt, 1932 NEW SYNONYMY
Hippia, of authors [not Möschler, 1878]
Elasmia Möschler, 1886 REVISED STATUS
Edema, of authors [not Walker, 1855]
Harma Walker, 1858a NEW SYNONYMY
Hippia, of authors [not Möschler, 1878]
Elymiotis Walker, 1857b
Bardaxima, of authors [not Walker, 1858b]
Cicynna Walker, 1858a
Edema, of authors [not Walker, 1855]
Gisara, of authors [not Schaus, 1901]
Nystalea, of authors [not Guenée, 1852]
Symmerista, of authors [not Hübner, 1821]
Euxoga Möschler, 1878
Ctianopha Schaus 1901, NEW SYNONYMY
Lysana, of authors [not Möschler, 1883]
Gopha Walker, 1862
Kryptokalos Weller, NEW GENUS
Heorta, of authors [not Walker, 1858c]
cilla Dognin, 1908 [Hippia] NEW COMBINATION
mitis Schaus, 1911 [Heorta]
oculata Dognin, 1909 [Lysana]
Lepasta Möschler, 1878
Antiopha, of authors [not Schaus, 1901]
Nystalea, of authors [not Guenée, 1852]
Lyricinus Weller, NEW GENUS
Etobesa, of authors [not Walker, 1865b]
Proelymiotis, of authors [not Schaus, 1901]
xylophasioides Butler, 1878 [Etobesa], NEW COMBINATION

Lysana Möschler, 1883
Proelymiotis, of authors [not Schaus, 1901]
Marthula Walker, 1856
Edema, of authors [not Walker, 1855]
Hippia Möschler, 1878, NEW SYNONYMY
Phastia, of authors [not Walker, 1862]
Pseudodryas, of authors [not Möschler, 1878]
Xanthia, of authors [not Guenée, 1852]
Notoplusia Schaus, 1901
Chadisra, of authors [not Walker, 1862]
Crinodes, of authors [not Herrich-Schäffer, 1855]
Rincodes Schaus, 1901, NEW SYNONYMY
Nystalea Guenée, 1852
Antiopha, of authors [not Schaus, 1901]
Congruia Dyar, 1908
Cyrrhesta Walker, 1857b
Eunystalea Grote, 1895
Heterocampa, of authors [not Doubleday, 1841]
Proelymiotis Schaus, 1901
Phedosia Möschler, 1878
Bardaxima, of authors [not Walker, 1858b]
Phyllopalpia Draudt, 1932
Antiopha, of authors [not Schaus, 1901]
Poresta Schaus, 1901, REVISED STATUS
Edema, of authors [not Walker, 1855]
Proelymiotis, of authors [not Schaus, 1901]
Nystalea, of authors [not Guenée, 1852]
Strophocerus, of authors [not Möschler, 1883]
Strophocerus Möschler, 1883
Antiopha, of authors [not Schaus, 1901]
Nystalea, of authors [not Guenée, 1852]
Poresta, of authors [not Schaus, 1901]

NYSTALEINAE [SENSU LATO]
Bahaia Dyar, 1924
Betola of authors [not Schaus 1901]
Dasylophia Packard, 1864
Drymonia, of authors [not Hübner, 1819]
Edema, of authors [not Walker, 1855]
Elymiotis, of authors [not Walker, 1857b]
Heterocampa, of authors [not Doubleday, 1841]
Oedemasia, of authors [not Packard, 1864]
Phalaena, of authors [not Linnaeus, 1758]
Proelymiotis of authors [not Schaus, 1901]
Symmerista, of authors [not Hübner, 1821]

Didugua Druce, 1891
Euharpyia Schaus, 1901
Lusura Walker, 1855
    Tifama Walker, 1855
    Chaetognatha Felder, 1874
Notela Schaus, 1901
Pentobesa Schaus, 1901
    Edema of authors [not Walker, 1855]
    Betola Schaus, 1901
    Nycterotis, of authors [not Felder, 1874]
    Proelymiotis of authors [not Schaus, 1901]
    Symmerista of authors [not Hübner, 1821]
    Tifama of authors [not Walker, 1855]
Symmerista Hübner, 1821
    Edema Walker, 1855

Table 2. List of other notodontid genera and species examined. Classification follows Forbes (1935), Weller (1989), Miller (1991), and Miller and Otero (1994).

Subfamily: Genus species
DIOPTINAE
Dioptini
Dioptis trailii Butler
Phryganidia californica Packard
Josiini
Erbessa unimacula (Warren)
Josia sp.
Scotura nervosa Schaus
DUDUSINAE
Dudusa sommeri (Hübner)
Crinodes bellatrix Stoll
Crinodes sp.
HEMICERATINI[1]
Antaea juturna Cramer
Apela strigatula Forbes
Apela sp.
Hapigia curvilinea Schaus
H. nodicornis Guenée
Hemiceras near *pallidula* Guenée
Hemiceras sp.

HETEROCAMPINAE
Heterocampini
Heterocampa astarte Doubleday
H. astartoides Benjamin
H. guttivitta (Walker)
Stauropini
Stauropus fagi (Linnaeus)
Tribal affiliation unknown
Chadisra bipars Walker
Chadisra sp.
Disphragis notabilis (Schaus)
D. tharis (Stoll)
Farigia sp.
Heorta roseoalba Walker
Litodonta hydromeli Harvey
Malocampa bolivari (Schaus)
Pamcoloma marita Schaus
Rhuda dimidiata (Herrich-Schäffer)
R. focula (Cramer)
R. splendens (Druce)
Rifargia lineata (Druce)
Rifargia near *mortis* Schaus
Rifargia near *onerosa* Schaus
Talmeca perplexa Schaus
Urgedra striata Druce

NOTODONTINAE
Dicranurini
Cerura vinula (Linnaeus)
Notodontini
Pheosia gnoma (Fabricius]
P. tremula (Clerck)

PHALERINAE
Datana ministra (Drury)
Nadata gibbosa (J.E. Smith)

PYGAERINAE
Clostera curtula (Linnaeus)

THAUMETOPOEINAE
Gazalina sp.
Thaumetopoeia processionea (Linnaeus)

INCERTAE SEDIS

Anurocampa mingens Herrich-Schäffer
Canodia difformis Herrich-Schäffer
Lirimiris lignitecta Walker[1]
Lirimiris sp.[1]
Lobeza Smithi Druce
Zelica myops (Felder)
Zelica zelica (Stoll)
Zelica sp.

[1] Miller (1991) places Hemiceratini and *Lirimiris* as *incertae sedis*

Table 3. Summary of specimens dissected. Classification follows Miller (1991)(M = male, F = female, g = genitalic preparation, w = whole body preparation).

Taxon	No. of Genera		No. of Species		No. of Preparations			
	Total	Examined	Total	Examined	M-g	F-g	M-w	F-w
Nystaleinae	31(25)[1]	31	253	170	309	147	34	9
Heterocampinae	37	30(3[2])	398[3]	34(4[2])	50	34	12	3
Hemiceratini	11	7	287	9	10	5	4	2
Dioptinae	40	3	400	3	4	5	3	3
Notodontinae	9[3]	1(2[2])	14[3]	2(2[2])	6	6	1	0
Dudusinae	7+[4]	2	93+[4]	3	3	1	1	0
Phalerinae	6+[4]	5	88+[4]	5	6	5	1	1
Pygaerinae	?[4]	1	?[4]	1	6	4	4	0
Lirimiris	1	1	16	2	2	1	0	0
Thaumetopoeinae	23	2	100	3	5	3	1	1

[1] 25 genera after revision (Weller, 1989)
[2] Old World taxa
[3] New World taxa only
[4] Estimates tentative or unavailable (= ?) (Miller 1991)

University Museum, Oxford University, Oxford, England; VOB, V.O. Becker, private collection, Brasilia, Brazil; ZMHB, Zoologisches Museum an der Humboldt-Universität zu Berlin, DDR-Germany (H.J. Hannemann). Figures list the museum collection and source slides or whole body preparation numbers (e.g., AMNH genitalia preparation SJW219).

Terminology

Terminology for genitalic structures follows Forbes (1948), Sibatani et al. (1954), Sibatani (1972), and Klots (1970), except where I propose new terms. Terminology for the tympanum follows Richards (1932), Forbes (1916), and Kiriakoff (1950a), with reinterpretations of some structures. A lexicon and definitions of terms applicable to notodontid morphology is provided.

Morphology and Discussion

Head (Figures 1-3)

The notodontid vertex is usually tightly scaled. Ocelli are present in some species (Forbes, 1948), but can be absent (e.g., *Litodonta hydromeli*: Heterocampinae). Often, a broad band of demelanized cuticle connects the ocelli across the vertex. In most species, the ocelli are located dorsal to the antennal scape and bordering the compound eyes (Fig. 1). The compound eyes are well developed, and the ocular index (frons width/ eye height) (Davis, 1975) ranges from 0.25 (*L. hydromeli*) to 1.0 (*Gazalina* sp.: Thaumetopoeinae) (Table 4). That is, *L. hydromeli* has very large eyes, and *Gazalina* sp. has very small eyes. Presumably, the ocular index and similar measures (eye width/frons width; Ferguson, 1985) reflect degree of night vision acuity. I have not surveyed intraspecific or intrageneric variation. Ferguson (1985) found that eye size may vary seasonally and geographically in arctiids.

The ventral border of the compound eyes and ventrum of the occiput often have long scales and hairs that partially cover the lower eye portion.

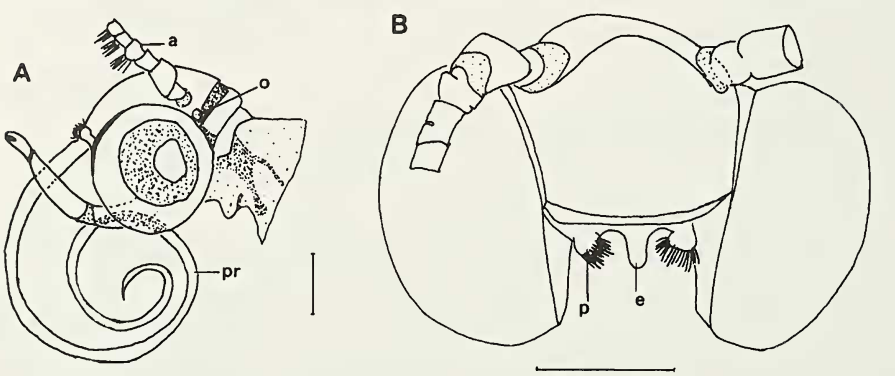

Figure 1. Descaled head of *Rifargia lineata* (NMNH 43,488, male). A. lateral view; B. frontal view. a = antenna, e = epipharynx, o = ocellus, p = pilifer, pr = proboscis. (Scale = 1.0 cm)